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Date July 16, 2002

AMENDMENT UNDER 37 C.F.R. §1.111		Attorney Docket	TGEN-001
Address to:	Assistant Commissioner for Patents	Confirmation No.	7852
Washington, D.C. 20231		First Named Inventor	Irena N. Merenkova
		Application Number	09/471,703
		Filing Date	December 23, 1999
		Group Art Unit	1634
		Examiner Name	Lisa Bennett Arthur
		Title	"Analysis of Nucleotide Polymorphisms at a Site"

Sir:

This amendment is responsive to the Office Action dated March 22, 2002 for which a three-month period for response was given making this response due on or before June 22, 2002. This amendment is accompanied by a Petition for a One Month Extension of Time, making this response due on or before July 22, 2002. In view of the amendments to the claims and the remarks put forth below, reconsideration and allowance are respectfully requested.

AMENDMENTS

IN THE CLAIMS

Cancel claims 39, 61-68 without prejudice as being drawn to a non-elected invention. Replace the pending claims with their correspondingly numbered claims below. Claims amended herein are indicated by text in parentheses.

- Bl 34. (Amended) A method for determining the identity of a polymorphic nucleotide in a target sequence having at least two known variants, comprising:
obtaining a sample comprising said target sequence;
hybridizing a primer upstream of said polymorphic nucleotide;
performing a first extension reaction with said hybridized primer in the absence of a deoxyribonucleoside triphosphate (dNTP) or ribonucleoside triphosphate (rNTP) complementary to said first known variant, but in the presence of at least one dNTP or rNTP complementary to said second known variant, wherein said dNTP or rNTP complementary to

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said second known variant is not detectably labeled or modified, and wherein the first extension reaction is performed in the absence of a dideoxynucleoside triphosphate (ddNTP);

performing a second extension reaction with said hybridized primer in the absence of a dNTP or rNTP complementary to said second known variant, but in the presence of at least one dNTP or rNTP complementary to said first known variant, wherein said dNTP or rNTP complementary to said first known variant is not detectably labeled or modified, and wherein the second extension reaction is performed in the absence of a ddNTP; and

analyzing the reaction products of said first extension reaction and said second extension reaction.

35. The method of claim 34 wherein a plurality of dNTPs or rNTPs is included in said first and second extension reactions.

36. The method of claim 34 wherein only one dNTP or rNTP is included in said first and second extension reactions.

37. The method of claim 34 wherein said primer hybridizes such that its 3' end is immediately upstream of the polymorphic base.

38. The method of claim 37 wherein one dNTP or rNTP is added.

40. The method of claim 34, wherein said target sequence is amplified *in vitro*.

41. The method of claim 34, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises determining the identity of the incorporated nucleotide which is complementary to said first known variant or said second known variant.

42. The method of claim 34, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises determining the length of said reaction products.

43. The method of claim 34, wherein said step of analyzing the reaction products of said first extension reaction and said second extension reaction comprises performing a technique selected from the group consisting of chromatography, capillary electrophoresis, microfluidic analysis, and slab gel electrophoresis.

44. The method of claim 34, wherein the reaction products are detected using high performance liquid chromatography.

45. The method of claim 34, wherein the reaction products are detected using capillary electrophoresis.

46. The method of claim 34, wherein the reaction products are detected using an intercalating agent.

47. The method of claim 46, wherein said intercalating agent is ethidium bromide.

48. The method of claim 46, wherein said intercalating agent is an unsymmetrical cyanine dye.

49. The method of claim 34, wherein the reaction products are detected using slab electrophoresis and ultraviolet light.

50. The method of claim 34, wherein the reaction products are detected using slab electrophoresis and a DNA-binding dye.

51. (Amended) The method of claim 34, wherein said target sequence having at least two known variants comprises a biallelic marker associated with genetic disorders.

52. The method of claim 34, wherein said sample containing a target sequence having at least two known variants is from a diploid organism.

53. The method of claim 34, wherein said first extension reaction is performed with a primer having a first length, and said second reaction is performed with a primer having a second length, said first and second lengths being selected such that said first primer and said second primer and any extension products thereof, can be distinguished from one another.

54. The method of claim 53, wherein the reaction products of said first and second extension reactions are analyzed separately.

55. The method of claim 53, wherein the reaction products of said first and second extension reactions are pooled for analysis.

83 56. (Amended) A method for screening a DNA sample for a plurality of target sequences having at least two known variants, comprising:

obtaining a sample comprising a plurality of known target sequences;
hybridizing a primer upstream of each of said target sequences, each primer having a length such that said primer and any extension product thereof can be distinguished from the other primers and any extension products thereof;
performing a plurality of extension reactions wherein each extension reaction contains a single free deoxyribonucleoside triphosphate (dNTP) or ribonucleoside triphosphate (rNTP) species complementary to one polymorphic nucleotide of said variant, wherein said single free dNTP or rNTP species is not detectably labeled or modified, and wherein the plurality of extension reactions are performed in the absence of a dideoxynucleoside triphosphate (ddNTP); and
analyzing the reaction products of each extension reaction.

57. The method of claim 56, wherein said target sequences being analyzed are associated with genetic disorders.

58. The method of claim 56, wherein said sample is from a diploid organism.

59. The method of claim 56, wherein the products of the extension reactions are analyzed separately.

60. The method of claim 56, wherein the products of the extension reactions are pooled for analysis.

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69. **(Amended)** A method for determining the identity of the polymorphic nucleotide in a target sequence having at least two known variants, comprising performing a primer extension reaction in the absence of a deoxyribonucleoside triphosphate (dNTP) or ribonucleoside triphosphate (rNTP) complementary to one of said polymorphic nucleotides but in the presence of at least one dNTP or rNTP complementary to the other polymorphic nucleotide, wherein said at least one dNTP or rNTP complementary to the other polymorphic nucleotide is not detectably labeled or modified, and wherein the extension reaction is performed in the absence of a dideoxynucleoside triphosphate (ddNTP); and detecting the reaction products of said extension reaction.

REMARKS UNDER 37 CFR § 1.111

Formal Matters

Claims 34-38, 40-60 and 69 are pending after entry of the amendments set forth herein.

Claims 34-60 and 69 were examined. All claims were rejected.

Please replace the pending claims with their correspondingly numbered clean version of the claims provided above.

Cancel claims 39, 61-68 without prejudice to renewal as being drawn to a non-elected invention. Applicants expressly reserve the right to pursue the subject matter of the canceled claims in a continuing application.

Claims 34, 51, 56, and 69 are amended. Support for the amendment to claims 34, 56, and 69 are found in the specification at, for example, page 3, lines 19-22; and in Example 1 (page 11, line 19 to page 14, line 19). Claim 51 is amended to correct a typographical error.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added.

Renumbering of claims

Applicants gratefully acknowledge the Examiner's renumbering of the claims as claims 34-69, and noting that the elected claims of Group I are claims 34-38 and 40-60 and 69, with claims 39, 61-68 withdrawn from consideration as being drawn to a non-elected invention. Applicants have canceled claims 39, 61-68 without prejudice.

Overview of the Claimed Invention

The invention involves methods for detection, or determining the identity, of a polymorphic nucleotide (e.g., claims 34 and 69) and or screening for a variant sequence (claim 56) by primer extension, where

- 1) the nucleotide (dNTP or rNTP) that is complementary to the polymorphic nucleotide (and thus would provide for primer extension in the presence of the appropriate polymorphic template) is not labeled or otherwise modified; and
- 2) the extension reaction is carried out in the absence of a terminator nucleotide (a dideoxynucleotide (ddNTP)).

As discussed below, none of the cited prior art either teaches or suggests a method for detection of a polymorphic nucleotide without a detectably labeled dNTP/rNTP that provides for extension and without the presence of a ddNTP terminator.

Rejection of the Claims Under §102(a): Hoogendoorn et al.

Claims 34-38, 40-44, 51-60 and 69 were rejected as being anticipated by Hoogendoorn et al. (Hum Genet (Jan 1999) 104:89-93). This rejection is traversed as applied and as it may be applied to the pending claims.

Hoogendoorn et al. discloses the use of a terminator nucleotide (ddNTP) in the extension reaction. The primer is extended by one nucleotide if the polymorphic nucleotide is present. A critical feature of the method of Hoogendoorn et al. is the use of ddNTP in the extension reaction. Specifically, at page 89, first full paragraph, Hoogendoorn et al. states:

In the presence of the appropriate dNTPs and ddNTPs, the primer is extended by one or more bases depending upon the sequence at the polymorphic site. . . . The alleles are then distinguished on the basis of size of the extended product.

(see also, Hoogendoorn et al., page 90, col. 3, second full paragraph thought fourth full paragraph). Nowhere does Hoogendoorn et al. suggest that this reaction can be performed in the absence of an appropriate ddNTP.

As noted above, the claimed methods involve extension reactions that are performed in the absence of a ddNTP. Because this feature is neither taught nor suggested by Hoogendoorn et al., the Examiner is respectfully requested to withdraw this rejection.

Rejection of the Claims Under §102(b): Nikiforov et al.

Claims 34-43, 45-60 and 69 were rejected as being anticipated by Nikiforov et al. (U.S. Pat. No. 5,679,524). This rejection is traversed as applied and as it may be applied to the pending claims.

Nikiforov et al. describes methods for detecting a single nucleotide polymorphism (SNP) by use of two primers (a "primer" and a "linker" oligonucleotide) which are hybridized adjacent to the SNP of the target sequence so as to be separated by a single nucleotide. Nikiforov et al. then disclose that the hybridized primer, linker, and target sequence are then subjected to an extension reaction in the presence of a polymerase, a ligase, and an appropriate dNTP. If the primer is extended to fill the gap between the primer and the linker, the product is ligated together to produce a product of primer-dNTP-linker in the presence of the appropriate target sequence.

Although Nikiforov et al. discloses variations of labeled components in the described detection methods (e.g., -- labeled dNTP, unlabeled primer and linker; -- labeled primer, unlabeled dNTP and linker; and -- labeled linker, unlabeled dNTP and primer, see col. 12, lines 3-9), Nikiforov et al. fails to disclose a method for detecting a polymorphic nucleotide where the dNTP is not detectably labeled or modified and the extension reaction is performed in the absence of a ddNTP. There is no explicit or implicit teaching in Nikiforov to this effect anywhere in the reference.

This assertion is supported in the examples provided in the Nikiforov et al. reference. In each of the examples, the extension reaction is performed using either 1) a selected labeled or unlabeled dNTP and each of the other ddNTPs (e.g., see Examples 1 and 2, particularly see col. 17, lines 45-53; and col. 18, lines 41-48); or 2) a selected detectably labeled dNTP and each of the other unlabeled dNTPs (see, col. 6, line 65 to col. 7, line 2; and Example 3, particularly col. 20, lines 12-15). This teaching of Nikiforov et al. thus indicates that one must perform the assay using either an unlabeled dNTP with a ddNTP or a detectably labeled dNTP and unlabeled dNTPs. There is no teaching or suggestion that the assay be performed with an unlabeled selected dNTP and in the absence of ddNTPs.

Therefore, the Examiner is respectfully requested to withdraw this rejection.

Rejection of the Claims Under §103(a): Kuppuswamy et al.

Claims 34-60 and 69 were rejected as being unpatentable over Kuppuswamy et al. This rejection is traversed as applied and as it may be applied to the pending claims.

The Office Action has cited Kuppuswamy et al. as disclosing a method for detecting a polymorphic nucleotide in a target sequence by primer extension reactions using either a radiolabeled dNTP that is complementary to the polymorphic nucleotide and unlabeled dNTPs that correspond to the non-polymorphic nucleotide, or vice versa. The reaction products were then resolved on a gel by electrophoresis so as to "obtain adequate resolution of the extended primer from the free nucleotide" (page 1144, col. 1, legend, Fig. 1). An autoradiograph was generated from the gel to detect incorporation of the detectably labeled dNTP.

The Office Action asserts that the method of Kuppuswamy et al. is different from the claimed methods "only in that Kuppuswamy et al. teaches that the dNTP is labeled and that detection of the single nucleotide extension products was performed by detecting the label and does not specifically teach detecting of a length difference by HPLC, capillary electrophoresis, microfluidic analysis or slab gel analysis." (Office Action, third full paragraph of section 6). The Office Action continues that such methods "are well known methods in the art for distinguishing oligonucleotides based upon a difference in length as small as a single nucleotide base." (see Office Action, fourth full paragraph of section 6). The Office Action then concludes that modification of the method of Kuppuswamy et al. to use unlabeled dNTPs and to detect extension on the basis of size differences would be obvious.

It is well-settled that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 21 USPQ2d 1941 (Fed. Cir. 1992), MPEP § 2143.01.

As summarized in MPEP §2142, it is well-settled that a *prima facie* case of obviousness requires:

- a) suggestion or motivation to combine and carry out, either in the cited references or in the knowledge generally available;
- b) a reasonable expectation of success in obtaining the claimed invention; and
- c) the prior art or combination of such, must teach all of the claim limitations

Each of these elements must be present for obviousness to be found.

Applicants respectfully submit that in the present case, element a) required by the law is absent. That is, there is no motivation to combine the method of Kuppuswamy et al., which uses a detectable dNTP, with a method for detection that requires discrimination of single nucleotide extension products by size. Kuppuswamy et al. provides a means to detect and discriminate between extension products -- the dNTP detectable label. The detectable label of the dNTP is critical to the method of Kuppuswamy et al., and is sufficient to discriminate between the presence or absence of the single nucleotide polymorphism. Kuppuswamy et al. uses no other detectable label, e.g., the primer for the extension reaction is not labeled.

Given the difficulties and labor involved in size-based discrimination -- particularly in the context of size-based discrimination of extension products that *differ by only a single nucleotide* -- the ordinarily skilled artisan would simply not be motivated to substituted the labeled dNTP with a size-based detection/discrimination method as asserted by the Office Action.

In view of the arguments above, the Examiner is respectfully requested to withdraw this rejection of the claims.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

Atty Dkt. No.:TGEN-001
USSN: 09/471,703

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number TGEN-001.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: July 16, 2002

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